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Conference Report:

Mutagenesis, speciation, and genome analysis in medaka

(January 20–21, 1994, held at National Institute of Basic Biology, Okazaki).

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The conference succeeded the highly successful meeting in 1992, "Present and future of medaka biology — molecular biology to field survey —". In the 1992 meeting, we comprehensively reviewed various fields of biology in which medaka had been used as an experimental animal, and discussed advantages of using the medaka in biological research. It was recognized that medaka showed the highest potential for use in genetic studies in each field of biology discussed and that it was meaningful in itself to understand how medaka has evolved in Asia and achieved speciation, since medaka lives together with us in the natural environment. As to the research of speciation, medaka is different from other experimental animals which are used only as models. Thus, our discussion at this conference was focused on genetics (mutagenesis), speciation, and genome analysis which forms the basis of these two subjects. The conference was organized by Drs. A. Shima (Tokyo Univ.) and K. Ozato (Kyoto Univ.), managed by Dr. Y. Nagahama (Natl. Inst. Basic Biol.), and supported by The Cooperative Research Program of the National Institute of Basic Biology. Fifty-five researchers attended the conference.

Session 1. Mutagenesis

Spontaneous mutagenesis in medaka (H. Tomita, Nagoya Univ.)

Medaka *Da* mutant and establishment of an ES-like cell line (Y. Wakamatsu, Kyoto Univ.)

Induced mutagenesis and genome analysis in medaka (A. Shima, A. Shimada and H. Wada, Tokyo Univ.)

Session 2. Speciation

Speciation of genus *Oryzias* in Asia (T. Iwamatsu, Aichi Univ. Edu.)

Medaka and its relatives viewed from DNA markers (M. Sakaizumi, Niigata Univ.)

Phylogenetic analysis using retroposons (N. Okada, Tokyo Inst. Technol.)

Session 3. Genome analysis

Analysis of medaka MHC Class II and tyrosinase genes (H. Hori, Nagoya Univ.)

Fugu genome and medaka genome (M. Tanaka, Natl. Inst. Basic Biol.)

Genome analysis of *C. elegans* (Y. Kohara, Natl. Inst. Genetics)

Tomita (Nagoya Univ.), who was nearing retirement from the university, reviewed his 30 years of research of spontaneous mutagenesis of medaka. He had collected and maintained 78 mutants including morphogenetic and color mutants, some of which were isolated in 1930. He brought a newly isolated mutant to the conference room, which lacked eyes, *eyeless*(*el*). His lecture which included references to his personal experiences as well as scientific data left a deep impression on the minds of the audience. Among the mutant stocks at Nagoya University, the *double anal fins* (*Da*) mutant has been known as a mutant of dorsal-ventral patterning. The name of *double anal fins* is derived from the fact that the dorsal fin in the mutant is similar to the anal fin in shape. It was reported that the ventralization of the dorsal structures was observed not only in the fins, but also in the pattern of melanophore arrangement, morphology of myotomes, and formation of finfolds (Y. Wakamatsu, Kyoto Univ.).

A new method for efficient induction of mutation was developed in medaka using radiation or a chemical mutagen (ENU). It was anticipated that large-scale mutagenesis will become possible in medaka by application of this method as it has in zebrafish (A. Shima, A. Shimada, and H. Wada). In addition to "orthodox genetics", the first step toward "reverse genetics" using ES cells has been achieved in medaka. It was reported that an

embryonic stem-like cell line was established in medaka, which exhibited pluripotent differentiation in culture (Y. Wakamatsu, Kyoto Univ.).

In genus *Oryzias,* 15 species have been identified, which are distributed specifically in Asia from India to Japan. Iwamatsu (Aichi Univ. Edu.) reviewed the origin and speciation of *Oryzias* on the basis of biogeography, karyotypes, and morphology. In the studies of the phylogenetic relationships among *Oryzias* species using DNA markers, Sakaizumi (Niigata Univ.) indicated that a sex chromosome-related DNA sequence was found specifically in the biarmed chromosome group containing four species and that a repeated sequence in mitochondrial DNA was found in three species except for *O. mekongensis* in this group. Thus, it was suggested that the biarmed chromosome group was monoplyletic and *O. mekongensis* first diverged in this group. He also emphasized that medaka was different from other laboratory animals in that the genetics of natural populations has been extensively studied.

The short interspersed element (SINE) is a repetitive sequence distributed widely in the genome of multicellular organisms and is considered to be a retroposon. Okada (Tokyo Inst. Technol.) reviewed his studies on the distribution and amplification of SINE in the process of salmonid fish evolution, showing that different types of SINE have been amplified and distributed at various stages of evolution. Distribution and amplification of SINE in *Oryzias* are interesting problems for elucidating phylogeny of this genus.

Although many medaka mutants have been collected, we have no linkage map to identify the mutant genes. Shima *et al.* (Tokyo Univ.) presented data of linkage analysis in medaka which was conducted using AP-PCR. They raised up 147 genetic markers by AP-PCR using 25 arbitrary primers, and identified 25 linkage groups. This was the first comprehensive linkage analysis in medaka and is expected to contribute to the construction of precise genetic maps. With regard to the structure of medaka tyrosinase gene, Hori (Nagoya Univ.) indicated that the tyrosinase gene of an albino mutant contained an insertional sequence, which was similar to that of transposable elements. His report produced much excitement, because no transposable elements had been known in vertebrates. It will be interesting to determine whether other mutants of medaka are also caused by insertion of this type of sequences.

C. elegans is one of the experimental animals in which genome analysis has made rapid progress. Kohara (Natl. Inst. Genetics) gave a lecture on methodology of genome analysis in *C. elegans,* which has a 100 Mb genome containing 15,000 genes. In addition to the conventional method, he introduced his unique strategy of the systematic analysis of cDNA clones to identify all *C. elegans* genes. His lecture was very exciting for attendees who intended to start genome analysis of medaka.

Now, small laboratory fish (zebrafish and medaka) are finding a place among the major experimental animals such as *C. elegans*, *Drosophila, Xenopus,* and mice. In this context, the conference was timely for discussing the future of medaka in biological research.